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Electronic Supplementary Information

Synthesis of DNA block copolymers with extended nucleic acid segments by enzymatic ligation: cut and paste large hybrid architectures

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1. Materials and Methods

1.1 Materials

All solvents and reagents were purchased from Sigma-Aldrich (Germany) and used without further purification, unless otherwise noted. The succinimide activated poly(ethylene glycol)s (PEG)s (Mn: 5000 g/mol and 20000 g/mol) were purchased from Nektar Therapeutics (USA). The dialysis membranes composed of regenerated cellulose (MWCO = 1000 g/mol and 8000 g/mol) and the phosphoramidites were obtained from Spectrum Laboratories Inc. (USA) and Link Technologies (UK), respectively. Thiol- and amino-functionalized oligonucleotides were synthesized by Biomers.net (Germany). The enzymes and buffers were obtained from Fermentas (Germany) and Roche Applied

Science (Germany). In all experiments, ultrapure water (18 MΩ) dispensed through 0.22 μm membrane filter from Millipore Inc. (USA) was used.

1.2 Sequences and synthesis of DNAs and their polymer hybrids

Table S1 ssDNA di- and triblock copolymers (**ssDB1-4** and **ssTB**, respectively) and their complementary sequences, with sticky ends and 5' phosphates, used in the ligation process.

Label	DNA sequence in 5' to 3'-direction	Mn of the synthetic polymer segment [g/mol]
ssDB1	PEG-CCTCGCTCTGCTAATCCTGTTA	5000
ssDB2	PEG-CCTCGCTCTGCTAATCCTGTTA	20000
ssDB3	PNIPAM-CCTCGCTCTGCTAATCCTGTTA	6000
ssDB4	PPO-CCTCGCTCTGCTAATCCTGTTA	6800
ssTB	3'-GGAGCGAGACGATTAGGACAAT-5'-PEG-5'-TAACAGGATTAGCAGAGCGAGG-3'	4000
cDNA1-S	P-CGGTTAACAGGATTAGCAGAGCGAGG	-
cDNA1-M	P-GGGATAACAGGATTAGCAGAGCGAGG	-
cDNA1-L	P-CTCATAACAGGATTAGCAGAGCGAGG	-
cDNA2-S	P-CGGTCCTCGCTCTGCTAATCCTGTTA	-
cDNA2-M	P-GGGACCTCGCTCTGCTAATCCTGTTA	-
cDNA2-L	P-CTCACCTCGCTCTGCTAATCCTGTTA	-

Note: P denotes a phosphorylated end of DNA for the ligation reaction. The triblock architecture has different DNA sequences from diblocks.

The synthesis of di- (**ssDB1**, **ssDB2**, **ssDB3** and **ssDB4** with the sequence 5'-CCT CGC TCT GCT AAT CCT GTT A-3') and triblock (**ssTB** with 5'-TAA CAG GAT TAG CAG AGC GAG G-3') copolymers was carried out through either solution or solid-phase synthesis, as previously reported by our group.¹⁻³

1.3 Equipment and Techniques

UV/Vis absorption and fluorescence spectra were recorded on a plate reader SpectraMax® M2 from Molecular Devices (USA). The solid phase synthesis of DNA block copolymer was carried out on an ÄKTA oligopilot 100 system from Amersham Pharmacia Biotech (Sweden) using standard phosphoramidite chemistry. The purification and characterization of ssDNA block copolymers were performed by polyacrylamide gel electrophoresis (PAGE). The ligation products were analyzed by agarose gel electrophoresis using ethidium bromide staining and transillumination.

2. Synthesis of dsDB and dsTB Copolymers

Preparation of linear dsDNA di- and triblock copolymers by ligation entailed the following reaction steps: **a)** restriction of the plasmid DNA pBR322 with the enzyme Alw26I (BsmAI) on three positions to prepare dsDNA segments for extension, **b)** hybridization of ssDNA block copolymers with the appropriate complementary sequences, including overhang segments, and their enzymatic ligation with the restricted DNA segments. The detailed molecular biology protocols and synthesis are described below.

2.1 Restriction of pBR322 with the enzyme Alw26I (BsmAI)

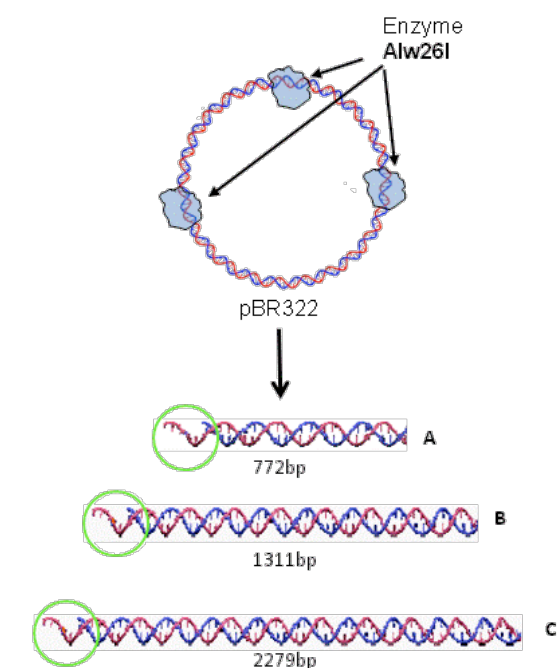


Fig. S1 Graphical representation of the DNA fragments of 772bp (A), 1322bp (B) and 2279bp (C) with sticky ends generated by restriction of pBR322 with the enzyme Alw26I.

The circular plasmid DNA pBR322 was digested by the enzyme Alw26I (BsmAI) at 37 °C for 3 h in a reaction buffer containing 1x Tango-Buffer, 20 ng/μl pBR322, and 1 u/μl Alw26I. The restricted products were purified after gel electrophoresis by QIAquick Gel Extraction Kit from Qiagen GmbH (Germany) using deionized water for eluting the DNA fragments. The digested products were characterized by agarose gel electrophoresis.

2.2 Hybridization of ssDNA blocks with sticky ends and ligation with the restriction products

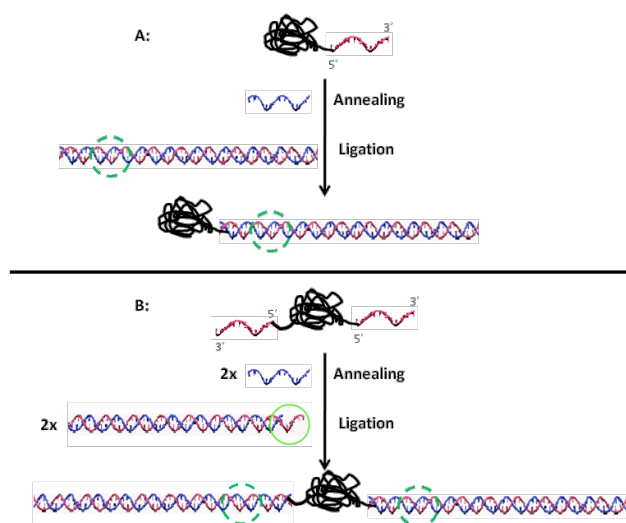


Fig. S2 Synthesis of dsDNA di- and triblock architectures via hybridization and ligation in one-pot synthesis. (A) dsDNA diblock architectures hybridized with **cDNA1s** (**dsDB1-4**) were extended with the corresponding DNA fragment (**S**, **M** or **L**). (B) dsDNA triblock architectures hybridized with **cDNA2s** (**dsTB**) were extended with the corresponding DNA fragment (**S**, **M** or **L**).

The synthesized ssDNA block copolymers **ssDB1**, **ssDB2**, **ssDB3** and **ssDB4** were hybridized with their 5'-phosphorylated complementary sequences **cDNA1-S**, **cDNA1-M** and **cDNA1-L**, and the ssDNA triblock architecture **ssTB** was hybridized with **cDNA2-S**, **cDNA2-M** and **cDNA2-L**; cDNAs labeled **S**, **M** or **L** have sticky ends complementary to the corresponding restriction products. This process was followed by ligation in the same reaction mixture.

Synthesis of extended diblock architectures (S-, M- or L-dsDBs): To obtain dsDNA diblock copolymers with extended nucleic acid segments, the hybridization and the ligation reactions were carried out in a one-pot synthesis in 200 μ l reaction mixtures containing 40 mM Tris-HCl, 10 mM MgCl_2 , 10 mM DTT, 5 mM ATP (pH 7.8), 0.25 u/ μ l T4 DNA ligase, 0.1 μ M **ssDB**, 0.2 μ M **cDNA1** and 0.5 μ M of the corresponding restriction product. The mixtures were incubated at 16 $^{\circ}\text{C}$ for 48 h.

Synthesis of extended triblock architectures (S-, M- or L-dsTBs): Synthesis of dsDNA triblock architectures was performed in 200 μ l reaction mixtures containing 40 mM Tris-HCl, 10 mM MgCl_2 , 10 mM DTT, 10 mM ATP (pH 7.8), 5u/ μ l T4 DNA ligase, 0.1 μ M **ssTB**, 0.4 μ M **cDNA2** and 1 μ M of the corresponding restriction product. The mixtures were incubated at 4 $^{\circ}\text{C}$ for 96 h.

Isolation of the products: After preparative agarose gel electrophoresis, the extended dsDNA di- and triblock products were purified by QIAquick Gel Extraction Kit from Qiagen GmbH (Germany) using deionized water for eluting the desired block architectures. The products were characterized by agarose gel electrophoresis.

3. AFM Imaging

Samples were diluted to make objects distinguishable on the mica surface due to buffer-induced aggregation. For instance, the material depicted in Fig. 2C is 80x diluted.

Atomic Force Microscopy (AFM). The images were recorded in liquid using a scanning force microscope (MultiMode, Nanoscope IIIa, Veeco Instruments, Santa Barbara, CA) operating in soft tapping mode. A piezoelectric J-scanner equipped with a thermal application controller (Veeco Instruments) was used.

Oxide-sharpened silicon nitride cantilevers (NP-S, Veeco Instruments; 115 μm long, 17 μm wide, 0.6 μm thick) with an integrated tip (nominal tip radius 10 nm; spring constant 0.32 N/m, and resonance frequency of 56 kHz in air) were used. The tips were cleaned with argon plasma and ethanol before measurements. MgCl_2 in ultrapure water was used to stabilize DNA samples on mica surfaces.

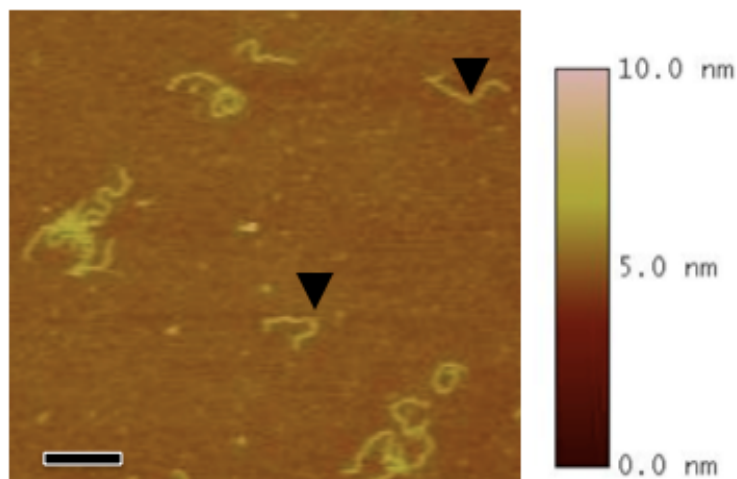


Fig. S3 AFM image of 10x-diluted extended triblock architecture **S-dsTB**. Observed kinks are marked by inverse triangles. Scalebar: 200 nm.

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4. Restriction Endonuclease Analysis of Extended Copolymers

Several dsDNA di- and triblock copolymers were further analyzed by a sequence-specific restriction endonuclease. As an example, the restriction analysis of dsDNA-*b*-PEG(20K) with a DNA segment of 795 bp and of dsDNA-*b*-PEG(4K)-*b*-dsDNA with DNA segments of 795bp is described below. As a control, non-polymer-modified DNA of 795 bp with the same sequence as the block copolymers was employed. The DNA di- and triblock copolymer and the dsDNA were digested by the enzyme *Dra*I at 37 °C for 3 h in the reaction buffer containing 33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate and 0.1 mg/ml BSA. The digested products were analyzed by agarose gel electrophoresis.

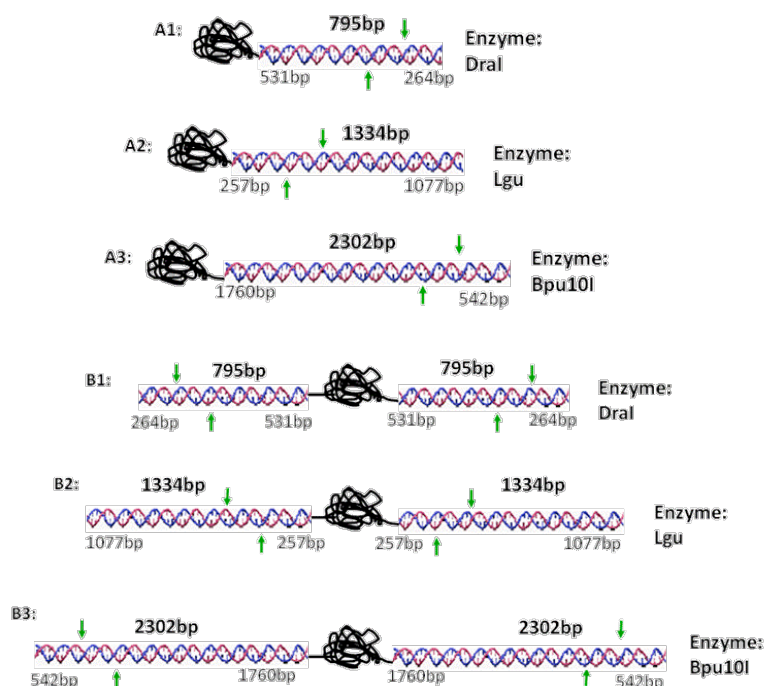


Fig. S4 Graphical representation of restriction endonuclease analysis of dsDNA di- and triblock copolymers. A1): digestion of 795bp dsDNA diblock by the enzyme DraI. A2): digestion of 1334bp dsDNA diblock by the enzyme Lgu. A3): digestion of 2302bp dsDNA diblock by the enzyme Bpu10I. B1): digestion of 795bp dsDNA triblock by the enzyme DraI. B2): digestion of 1334bp dsDNA triblock by the enzyme Lgu. B3): digestion of 2302bp dsDNA triblock by the enzyme Bpu10I.

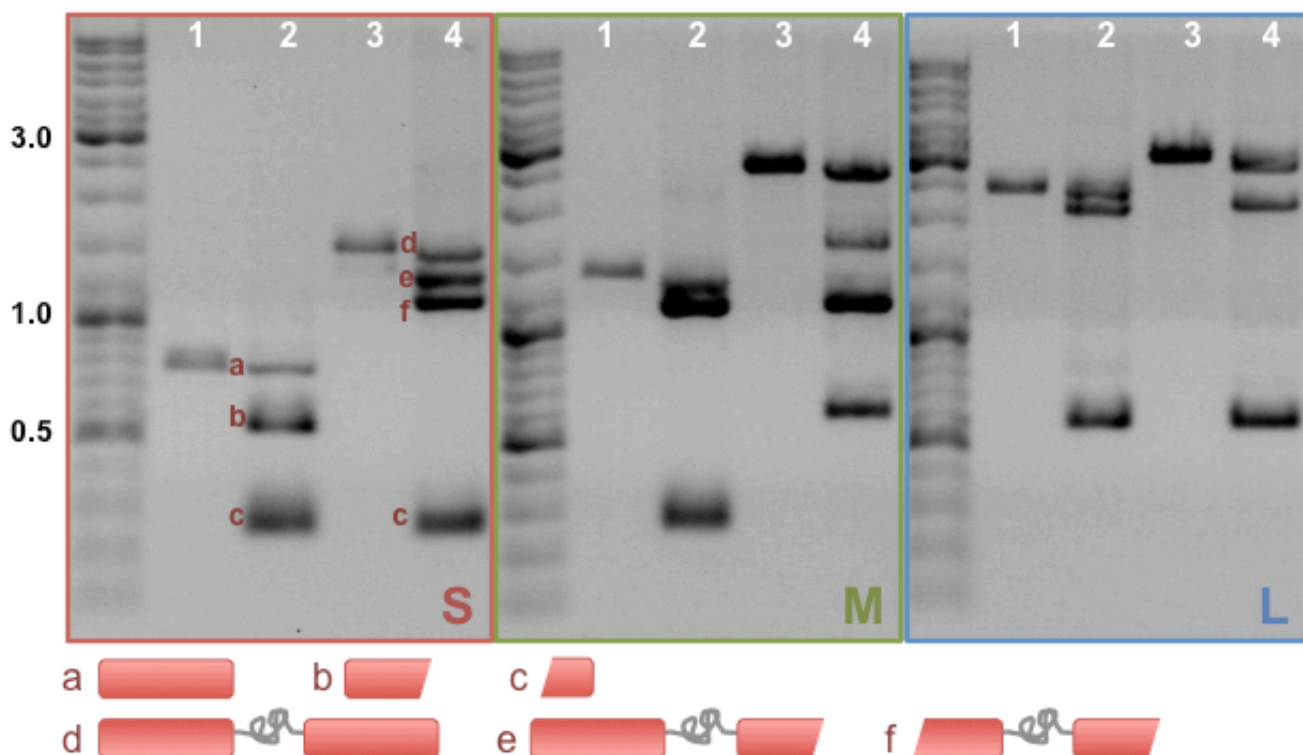


Fig. S5 Restriction analysis of the extended triblock architectures by PAGE (top). The order of samples in each group: 10 pristine dsDNA without the restriction enzyme (lane 1), its digested dsDNA with the enzyme (lane 2), corresponding **dsTB** architectures without (lane 3) and with (lane 4) the enzyme. Note that Fig. 3B is a subset of this gel image. Graphical representations of the gel bands in group S are presented as a-f (bottom).

Analysis of gel images: Agarose gel images after enzymatic restriction were analyzed with a public domain Java image processing program, imageJ (NIH).⁴ High-quality images recorded by the camera in the gel document system were processed with imageJ, resulting in graphs of band intensities as a function of electrophoretic mobility; see Fig S6 for an example.

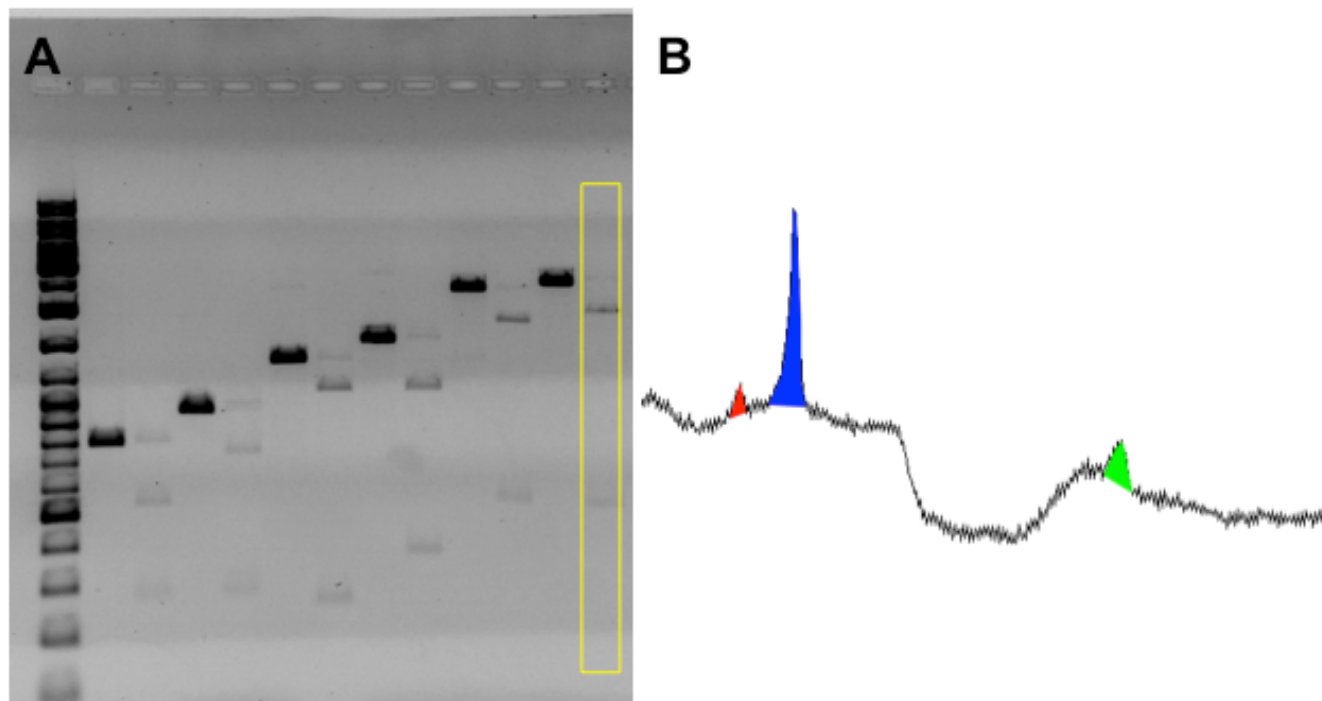


Fig. S6 A gel image analysis of endonuclease restriction results (A) A raw gel image, identical to Fig. 3A, of restriction analysis by enzymes. See Fig 3. for the description of samples. (B) Analysis of marked lane (yellow) with imageJ software. Using image processing software, each band area (intensity as a function of gel mobility) was marked in order to compare enzyme efficiencies.

Table S2 Enzyme efficiencies with pristine dsDNA and diblock architectures.

Starting material	Restriction enzyme	Area of low molecular weight fragment	Area of high molecular weight fragment	Area of starting material	Total area	Efficiency (%)
S-dsDB2	DraI	499	1373	191	2063	91
S-dsDNA (control)	DraI	376	1300	135	1811	93
M-dsDB2	LgU	903	2381	317	3601	91
M-dsDNA (control)	LgU	863	2038	212	3113	93
L-dsDB2	Bpu10I	684	1961	675	3320	80
L-dsDNA (control)	Bpu10I	1416	2558	652	3626	82

Note: All sample loadings and electrophoresis were performed in identical concentrations and gel conditions. Area units are pixels of integrated band area from images.

A similar analysis was performed for triblock architectures. However, the result is not directly comparable because pristine dsDNA is not a suitable control sample for triblocks due to their different architectures.

Table S3 Gel image analysis of digested pristine ds DNAs and triblock architectures.

Starting material	Restriction enzyme	Area (%) of starting material	Area (%) of single digestion	Overall efficiency (%)
S-dsTB	DraI	19	23	58
S-dsDNA (control)	DraI	10	N/A	N/A
M-dsTB	LgU	25	12	63
M-dsDNA (control)	LgU	28	N/A	N/A

Note: Overall efficiency = 100 – (starting material + single digestion). The gel image of **L-dsTB** was not analyzed due to the poor resolution of individual bands in the high Mw range (lanes L2 and L4 in Fig. S6).

5. References and Notes

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